

IN THE SPECIFICATION:

Please replace the paragraph starting at page 1, line 18, with the following amended paragraph:

This application is a divisional application of U.S. Patent Application Serial No. 08/971,344 (filed November 17, 1997) now abandoned, which is a continuation application of U.S. Patent Application Serial No. 08/216,538 (filed March 23, 1994) now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 08/145,145 (filed Nov. 3, 1993) and abandoned January 11, 1996).

Please replace the paragraph starting at page 12, line 21 to page 13, line 18, with the following amended paragraph:

Nucleic acid molecules having the sequence complementary to that of an immediately 3'-distal invariant sequence of a SNP can, if extended in a "template-dependent" manner, form an extension product that would contain the SNP's polymorphic site. An preferred example of such a nucleic acid molecule is a nucleic acid molecule whose sequence is the same as that of a 5'-proximal invariant sequence of the SNP. "Template-dependent" extension refers to the capacity of a polymerase to mediate the extension of a primer such that the extended sequence is complementary to the sequence of a nucleic acid template. A "primer" is a single-stranded oligonucleotide or a single-stranded polynucleotide that is capable of being extended by the covalent addition of a nucleotide in a "template-dependent" extension reaction. In order to possess such a capability, the primer must have a 3'-hydroxyl terminus, and be hybridized to a second nucleic acid molecule (i.e. the "template"). A primer is typically 11 bases or longer; most preferably, a primer is 20 bases, however, primers of shorter or greater length may suffice. A "polymerase" is an

enzyme that is capable of incorporating nucleoside triphosphates to extend a 3'-hydroxyl group of a nucleic acid molecule, if that molecule has hybridized to a suitable template nucleic acid molecule. Polymerase enzymes are discussed in Watson, J. D., *In: Molecular Biology of the Gene*, 3rd Ed., W. A. Benjamin, Inc., Menlo Park, Calif. (1977), ~~which reference is incorporated herein by reference~~— and similar texts. Other polymerases such as the large proteolytic fragment of the DNA polymerase I of the bacterium *E. coli*, commonly known as "Klenow" polymerase, *E. coli* DNA polymerase I, and bacteriophage T7 DNA polymerase, may also be used to perform the method described herein. Nucleic acids having the same sequence as that of the immediately 3' distal invariant sequence of a SNP can be ligated in a template dependent fashion to a primer that has the same sequence as that of the immediately 5' proximal sequence that has been extended by one nucleotide in a template dependent fashion.

Please replace the paragraph starting at page 23, line 26 to page 24, line 3, with the following amended paragraph:

The direct analysis of the sequence of an SNP of the present invention can be accomplished using either the "dideoxy-mediated chain termination method," also known as the "Sanger Method" (Sanger, F., *et al.*, *J. Molec. Biol.* 94:441 (1975)) or the "chemical degradation method," "also known as the "Maxam-Gilbert method" (Maxam, A.M., *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 74:560 (1977) —, ~~both references herein incorporated by reference~~—. Methods for sequencing DNA using either the dideoxy-mediated method or the Maxam-Gilbert method are widely known to those of ordinary skill in the art. Such methods are, for example, disclosed in Sambrook, J. *et al.*, *Molecular Cloning, a Laboratory Manual, 2nd Edition. Cold Spring Harbor Press*, Cold Spring Harbor, N.Y. (1989), and in Zyskind, J. W., *et al.*, *Recombinant DNA Laboratory Manual, Academic Press. Inc.*, New York (1988), —, ~~both references herein incorporated by reference~~—.

Applicant: Goelet
U.S. Serial No.: 09/846, 863
Filing Date.: May 1, 2001
Amendment
Page 4 of 29

Please replace the paragraph starting at page 25, lines 5-12, with the following amended paragraph:

Most preferably, such single-stranded molecules will be produced using the methods described by Nikiforov, T. (U.S. patent application Ser. No. 08/005,061, ~~-herein incorporated by reference-, now abandoned~~). In brief, these methods employ nuclease resistant nucleotides derivatives, and incorporates such derivatives, by chemical synthesis or enzymatic means, into primer molecules, or their extension products, in place of naturally occurring nucleotides.

Please replace the paragraph starting at page 25, lines 19-31, with the following amended paragraph:

Phosphorothioate deoxyribonucleotide or ribonucleotide derivatives (e.g. a nucleoside 5'-O-1-thiophosphate) are the most preferred nucleotide, derivatives. Any of a variety of chemical methods may be used to produce such phosphorothioate derivatives (see, for example, Zon, G. *et al.*, *Anti-Canc. Drug Des.* 6:539-568 (1991); Kim, S. G. *et al.*, *Biochem. Biophys. Res. Commun.* 179:1614-1619 (1991); Vu, H. *et al.*, *Tetrahedron Lett.* 32:3005-3008 (1991); Taylor, J. W. *et al.*, *Nucl. Acids Res.* 13:8749-8764 (1985); Eckstein, F. *et al.*, *Biochemistry* 15:1685-1691 (1976); Ott, J. *et al.*, *Biochemistry* 26:8237-8241 (1987); Ludwig, J. *et al.*, *J. Org. Chem.* 54:631-635 (1989) ~~, all herein incorporated by reference~~). Phosphorothioate nucleotide derivatives can also be obtained commercially from Amersham or Pharmacia.

Please replace the paragraph starting at page 34, lines 1-8, with the following amended paragraph:

In one preferred embodiment, an oligonucleotide having a sequence that is complementary to an immediately distal sequence of a polymorphism is prepared using

the above-described methods (and preferably that of Nikiforov, T. (U.S. Patent Application Serial. No. 08/005,061, now abandoned). The terminus of the oligonucleotide is attached to the solid support, as described, for example by Goelet, P. et al. (PCT Application WO 92/15712), such that the 3'-end of the oligonucleotide can serve as a substrate for primer extension.

Please replace the paragraph starting at page 51, lines 13-23, with the following amended paragraph:

To obtain single-stranded template for use with solid-phase immobilized primer, either of two methods may be used. First, the amplification may be mediated using primers that contain 4 phosphorothioate-nucleotide derivatives, as taught by Nikiforov, T. (U.S. patent application Ser. No. 08/005,061, now abandoned). Alternatively, a second round of PCR may be performed using "asymmetric" primer concentrations. The products of the first reaction are diluted {fraction (1/1000)} in a second reaction. One of the second round primers is used at the standard concentration of 2 M while the other is used at 0.08 M. Under these conditions, single stranded molecules are synthesized during the reaction.